Polymerase Chain Reaction (PCR) Protocol

1. Carefully label a 0.2 ml PCR microfuge tube.
    a. NOTE: Handle the microfuge tube with care as it cracks easily.
    b. NOTE: Keep the Q5 master mix and primers on ice at all times.
1. Add 12.5 µL Q5 2x Master mix to the PCR microfuge tube.
2. Add 2.0 µL of the DNA Template to the microfuge tube.
3. Add 8 µL DepC Water to the microfuge tube.
4. Add 1.25 µL Primer A (TBD) and 1.25 µL Primer B (TBD) to the microfuge tube. (The combination of the two constitutes the working mix)
5. Gently pipette up and down to mix the solution.
6. After mixing, place your microfuge tube in the thermal cycler. The full process (~98 min), which is performed, is outlined below.
    a. Hot Start, 98°C, 3 min
    b. Denaturation, 98°C, 30sec
    c. Annealing, ~67°C, 30sec (Check the melting temperatures for the primers)
    d. Extension, 72°C, 2min
    e. Repeat 2-4 30 times
    f. Final Extension, 72°C, 5min
    g. Hold, 4°C, Infiniti
7. 8. Store your PCR product at -20°C.